

Synergistic Association of Genetic Variants with Environmental Risk Factors in Susceptibility to Essential Hypertension

Ana Célia Sousa,¹ Maria I. Mendonça,¹ Andreia Pereira,¹ Sara Gouveia,¹ Ana I. Freitas,^{1,2}
Graça Guerra,^{1,2} Mariana Rodrigues,¹ Eva Henriques,¹ Sónia Freitas,¹ Sofia Borges,¹
Décio Pereira,¹ António Brehm,² and Roberto Palma dos Reis³

Aims: Essential hypertension (EH) is a disease in which both environment and genes have an important role. This study was designed to identify the interaction model between genetic variants and environmental risk factors that most highly potentiates EH development.

Methods: We performed a case–control study with 1641 participants (mean age 50.6 ± 8.1 years), specifically 848 patients with EH and 793 controls, adjusted for gender and age. Traditional risk factors, biochemical and genetic parameters, including the genotypic discrimination of 14 genetic variants previously associated with EH, were investigated. Multifactorial dimensionality reduction (MDR) software was used to analyze gene–environment interactions. Validation was performed using logistic regression analysis with environmental risk factors, significant genetic variants, and the best MDR model.

Results: The best model indicates that the interactions among the *ADD1* rs4961 640T allele, diabetes, and obesity (body mass index ≥ 30) increase approximately four-fold the risk of EH (odds ratio = 3.725; 95% confidence interval: 2.945–4.711; $p < 0.0001$).

Conclusion: This work showed that the interaction between the *ADD1* rs4961 variant, obesity, and the presence of diabetes increased the susceptibility to EH four-fold. In these circumstances, lifestyle adjustment and diabetes control should be intensified in patients who carry the *ADD1* variant.

Keywords: genes, *ADD1*, environmental risk factors, obesity, MDR software, diabetes

Introduction

ESSENTIAL HYPERTENSION (EH) is a complex and multifactorial disease resulting from multiple environmental and genetic factors (Carretero and Oparil, 2000; Kuneš and Zicha, 2009; Joseph *et al.*, 2013).

Both clinical and experimental studies have already identified plausible mechanisms underlying the development of EH: increased activity of the sympathetic nervous system, overactivity of the renin–angiotensin aldosterone system, dysfunction of the vascular endothelium, thrombogenesis, and impairment of the pressure–natriuresis mechanism leading to salt retention and elevated peripheral resistance, among others (Oparil *et al.*, 2003). All these systems are under strict genetic control, and this is the cause that some individuals are more susceptible to EH than others.

The majority of currently available knowledge has greatly benefited by mapping the genes responsible for Mendelian forms of hyper- and hypotension (Lifton *et al.*, 2001) and studying rodent models with various blood pressure (BP) affecting phenotypes (Okamoto and Aoki, 1963; Cowley, 2006). These association studies have successfully identified multiple interacting molecular pathways that are involved in the determination of a subject's BP. Consequently, genes coding for the components of these molecular pathways have been targeted for the identification of genetic variations affecting interindividual differences in BP levels (Mein *et al.*, 2004; Ji *et al.*, 2008). However, results of a large number of association studies conducted with BP traits have been inconsistent.

Genome-wide association studies (GWAS) have emerged as a novel alternative to explore simultaneously a large number of genomic loci for associations with a phenotypic trait. This

¹Unidade de Investigação, Hospital Doutor Nélcio Mendonça, Funchal, Portugal.

²Laboratório de Genética Humana, Universidade da Madeira, Funchal, Portugal.

³Faculty of Medical Sciences, New University of Lisbon, Lisboa, Portugal.

method has shown a great promise in determining common polymorphisms responsible for several complex phenotypes like diabetes, stroke, and coronary artery disease (Altshuler *et al.*, 2008). However, GWAS have not produced clear results in respect of hypertension-associated gene polymorphisms (Wellcome Trust Case Control Consortium, 2007; Levy *et al.*, 2007; Ehret *et al.*, 2008). Although it has identified numerous loci associated with BP traits (Levy *et al.*, 2009), they only explain a small proportion of interindividual BP variability. The 29 loci identified by GWAS and reported by the International Consortium of Blood Pressure accounts for about 1% of BP variation in the general population (Ehret *et al.*, 2011).

Knowing the universal nature of epistasis in determining susceptibility to complex disease, one possible way to approach these complex conditions is to examine the joint impact of multiple genes involved in particular cellular or physiological pathways along with several environmental risk factors underlining it. Moreover, many clinical and experimental studies have bypassed important environmental factors that can affect BP development and progression (Zuoguang *et al.*, 2013).

In these circumstances, in complex diseases, like EH, more than to evaluate the polymorphisms one by one, it is important to evaluate several polymorphisms together with environmental factors.

Objective

The goal of the current study is to evaluate the best interaction model between 14 single nucleotide polymorphisms (SNPs) associated to EH and several environmental risk factors, in predicting the risk of EH in the population of Madeira Island (Portugal).

Methods

Study population

All subjects participating in the present study were selected from the Internal, General, and Familiar Medicine from our Hospital.

This study received approval from the ethics committee of the Funchal Hospital Center and was performed in conformity with the guidelines outlined in the Declaration of Helsinki statement. Written informed consent was obtained from each participant, including explicit permission for the DNA analyses and the collection of relevant clinical data.

Study design

A case-control study was performed with a cohort of 1641 individuals (50.6 ± 8.1 years; 49.9% male), namely 848 patients diagnosed with EH (mean age 50.8 ± 8.1; 51.8% male) and 793 normotensive controls (mean age of 50.3 ± 8.2; 47.9% male). Cases and controls were matched for sex and age.

EH was considered when patients, at the entry into this study, were already diagnosed and/or had been on antihypertensive medication for more than 3 months or newly diagnosed hypertensives with systolic blood pressure (SBP)/diastolic blood pressure (DBP) ≥ 140/90 mmHg measured on at least three occasions (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003). The normotensive controls had never been treated

with antihypertensive medication and presented a SBP/DBP < 140/90 mmHg.

BP was measured after 10 min of resting in the right arm, using a standard Welch Allyn sphygmomanometer (phases I through V). The average of three readings taken 2 min apart was recorded (European Society of Hypertension-European Society of Cardiology Guidelines Committee, 2003).

Data collection

Data recorded from each subject comprised demographic, clinical profile, and traditional risk factors (gender, age, body mass index [BMI], sedentary lifestyle, alcohol and smoking habits, and diabetes).

BMI was defined as body mass divided by the square of the body height, universally expressed in units of kg/m², with obesity defined as a BMI ≥ 30 (National Institutes of Health, National Heart, Lung, and Blood Institute North American Association for the Study of Obesity, 2000). Sedentary lifestyle was considered when individuals practiced < 150 min/week of moderate activity or 75 min of vigorous activity (The Department of Health, Government of Australia, 2014). Alcohol consumption was considered as a risk factor when individuals consumed 70 g of alcohol per week for more than 1 year. Moreover, individuals who smoked ≥ 70 cigarettes per week for more than 1 year were defined as “smokers” (Ji *et al.*, 2013).

Subjects were classified as being diabetic when taking oral antidiabetic medication or insulin or if their fasting plasma glucose was higher than 7.0 mmol/L or 126 mg/dL (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003).

Biochemical analysis

Blood samples were extracted after 14–16 h fasting. Biochemical analyses were performed in the Central Laboratory of the Hospital, according to standard techniques. To determine total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, triglycerides, and glucose, blood samples were placed in dry tubes, centrifuged half an hour later at 3500g and subsequently quantified by an enzymatic technique using a “AU 5400” (Beckman Coulter) auto analyzer. Biochemical markers such as lipoprotein-a, apolipoprotein B, and high-sensitivity C-reactive protein (hs-CRP) were quantified by immunoturbidimetry using an AU 5400 (Beckman Coulter) automatic system. To measure fibrinogen, samples were placed in a tube containing sodium citrate, and measurements were taken with an ACL TOP 700 automatic analyzer.

SNP selection

SNPs were selected either from GWAS or candidate gene association studies for *loci* biologically plausible to EH or BP, specifically: *ACE* rs4340, *ACE* rs4343, *AT1R* rs5186, *AGT* rs699, *AGT* rs4762, *ADD1* rs4961, *CYP11B2* rs1799998, *CYP17A1* rs11191548, *ADRB1* rs1801253, *ADRB2* rs1042713, *GNB3* rs5443, *AT2B1* rs2681472, *SLC4A2* rs2303934, and *SCNN1G* rs5718.

Entering criteria included genes with a minor allele frequency (MAF) > 5%. Genes in Hardy-Weinberg disequilibrium ($p < 0.0036$, after Bonferroni correction) were automatically excluded.

Genetic analyses

Genetic analyses were done at the Human Genetics Laboratory of the University of Madeira. Genomic DNA was extracted from 80 µL of peripheral blood using a standard phenol–chloroform method. A TaqMan allelic discrimination assay for genotyping was performed using labeled probes and primers pre-established by the supplier (TaqMan SNP Genotyping Assays; Applied Biosystems).

Quality check of TaqMan genotyping technique was maintained by the inclusion of one nontemplate control in each plate of 96 wells. Also, all SNP TaqMan assays had blind duplicates accounting for 20% of all samples. Some SNP genotypes were randomly confirmed by conventional direct DNA sequencing, as 10–15% of all samples were reamplified for sequencing.

All reactions were done on an Applied Biosystems 7300 Real-Time PCR System and genotypes were determined using the 7300 System SDS Software (Applied Biosystems, Foster City, CA) without any prior knowledge of individual's clinical data.

Statistical analyses

Genotypic frequencies were determined from observed counts and compared by Chi-square analysis. Representative samples were judged by comparing the genetic polymorphism frequency and Hardy–Weinberg equilibrium was tested at each locus on a contingency table of observed versus predicted genotype using the Chi-squared test.

Continuous and categorical variables were compared between the two groups by the unpaired Student's *t*-test, Mann–Whitney, and the Chi-squared test when appropriate. A two-tailed *p* value of <5% was considered statistically significant, whereas a value of *P*_B (PBonferroni divided by total number of comparisons) was considered significant after the Bonferroni correction.

Five statistical genetic models were tested, namely dominant, recessive, additive, multiplicative, and codominant. The relative risk factor was evaluated using odds ratio (OR) and 95% confidence interval (CI) for each model.

Interactions between variants significantly associated with EH and five environmental risk factors for EH (sedentary lifestyle, BMI, diabetes, smoking, and alcohol habits) were analyzed by using multifactorial dimensionality reduction (MDR) 3.0.2 software (Guy *et al.*, 2010; Yang *et al.*, 2015). The best combination pattern was searched on the principle

that both cross-validation consistency and test balance accuracy were maximized to evaluate the interaction of the genetic polymorphism with each other and with the five environmental risk factors, in relation to EH.

To test the validity of MDR method, we further conducted a classical logistic regression analysis that included the five environmental risk factors and the best MDR model.

Statistical analyses were performed using the Statistical Package for the Social Sciences Software version 19.0 (IBM, Armonk, NY).

Results

Characteristics of the population

Baseline characteristics of our population are listed in Table 1. There were no significant differences between cases and controls in terms of age and sex. When compared with controls, patients with EH had higher alcohol consumption (*p*=0.008), BMI (*p*<0.0001), obesity (*p*<0.0001), diabetes (*p*<0.0001), SBP (*p*<0.0001), DBP (*p*<0.0001), and heart rate (*p*<0.0001) (Table 1). Controls had more smoking habits in relation to hypertensive subjects, with statistical significance (*p*<0.0001).

Biochemical analysis of the population is shown in Table 2. Comparatively to controls, hypertensive patients had significantly higher levels of hemoglobin (*p*<0.0001), leucocytes (<0.0001), fibrinogen (<0.0001), glucose (<0.0001), triglycerides (<0.0001), apolipoprotein B (*p*<0.0001), and hs-CRP (<0.0001). On the other hand, HDL cholesterol showed higher values in controls, with a *p*<0.0001 (Table 2).

Genetic polymorphisms and EH risk

Risk prediction of 14 polymorphisms related to EH was evaluated under five genetic models of inheritance. Table 3 shows the results obtained from the best genetic model for each SNP. All variants were in Hardy–Weinberg equilibrium (Table 3). However, *SLC4A2* rs2303934 was excluded due its low MAF (3.2) (Table 3). The remaining 13 genetic variants were included for further analysis.

Only two showed a statistically significant increase in EH risk (*p*<0.05), namely, the *ADD1* rs4961 variant in the recessive model (OR=2.379; 95% CI: 1.135–4.985; *p*=0.018) and the *GNβ3* rs5443 variant in the dominant model (OR=1.275; 95% CI: 1.042–1.559; *p*=0.018) (Table 3). Additionally,

TABLE 1. BASELINE CHARACTERISTICS OF THE POPULATION

Variables	Total (n=1641)	Hypertensives (n=848)	Controls (n=793)	p Value
Age, years	50.6 ± 8.1	50.8 ± 8.1	50.3 ± 8.2	0.212
Male sex, n (%)	819 (49.9)	439 (51.8)	380 (47.9)	0.119
Sedentary life, n (%)	887 (54.1)	476 (56.1)	411 (51.8)	0.080
Alcohol, n (%)	627 (38.2)	350 (41.3)	277 (26.6)	0.008
Smoking, n (%)	375 (22.9)	164 (19.3)	211 (26.6)	<0.0001
BMI, kg/m ²	27.7 ± 4.9	29.2 ± 5.2	26.2 ± 4	<0.0001
Obesity, n (%)	441 (26.9)	326 (38.4)	115 (14.5)	<0.0001
Diabetes, n (%)	169 (10.3)	134 (15.8)	35 (4.4)	<0.0001
SBP, mmHg	134.4 ± 20.4	147.2 ± 18.9	120.7 ± 10.9	<0.0001
DBP, mmHg	84.5 ± 12.1	91.1 ± 11.7	77.4 ± 7.7	<0.0001
Heart rate, bpm	72.1 ± 11.8	73.1 ± 12.2	71 ± 11.2	<0.0001

Continuous variables are expressed by mean ± standard deviation.

Statistically significant for *p*<0.05.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; bpm, beats per minute.

TABLE 2. BIOCHEMICAL CHARACTERISTICS OF THE POPULATION

Variables	Total (n=1641)	Hypertensives (n=848)	Controls (n=793)	p Value
Hemoglobin, g/dL	14.3 (9.6–18.2)	14.4 (9.6–18.2)	14.2 (10.1–17.6)	<0.0001
Platelets, 10 ³ /μL	229 (23–664)	233 (23–664)	227 (65–544)	0.206
Leucocytes, 10 ³ /μL	6.4 (2.1–18)	6.6 (2.9–18)	6.2 (2.1–16.6)	<0.0001
Fibrinogen, mg/dL	362 (179.2–874)	371 (179.2–874)	355.3 (224–688)	<0.0001
Glucose, mg/dL	95 (66–364)	98 (70–360)	93 (66–364)	<0.0001
Lipoprotein (a), mg/dL	16.3 (0.6–236)	16.6 (0.6–236)	16.2 (0.8–198.2)	0.730
Cholesterol, mg/dL	207 (107–370)	208.5 (115–344)	206 (107–370)	0.193
HDL, mg/dL	48 (17.2–111.7)	46.9 (17.2–103)	49 (20.8–111.7)	<0.0001
LDL, mg/dL	131 (37.7–269)	131 (37.7–269)	131 (42–260)	0.944
Triglycerides, mg/dL	110 (21–1098)	119 (29–1098)	99 (21–688)	<0.0001
Apolipoprotein B, mg/dL	104 (3.9–232)	106.6 (5.1–205)	99.7 (3.9–232)	<0.0001
hs-CRP, mg/dL	0.22 (0.01–18.51)	0.25 (0.02–13.1)	0.19 (0.01–18.51)	<0.0001

Biochemical variables are presented by median (minimum–maximum).

Statistically significant for $p < 0.05$.

HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein.

the *ACE* rs4343 was marginally significant being at the threshold of significance ($p = 0.053$) with an OR of 1.148 (95% CI: 0.999–1.321) (Table 3).

Gene–environment interactions and the EH risk

MDR software was employed to analyze the interaction between the three mentioned variants (*ADD1* rs4961, *GNB3* rs5443, and *ACE* rs4343) and five nongenetic risk factors for EH (sedentary lifestyle, obesity, diabetes, smoking, and alcohol habits) (Table 4). The best association model included diabetes, obesity, and *ADD1* rs4961 polymorphism, which showed a maximal testing accuracy of 0.638 and a cross-validation consistency of 10 out of 10 (Table 4). These results indicate that *ADD1* rs4961 interacts with diabetes and obesity in a synergistic way with a 3.725-fold greater risk of EH

(Table 4). Consequently this model was chosen as the overall best MDR model, which was significant at a level of 0.0002, indicating overall a better performance by 2 out of 10,000 permutations and was thus unlikely under the null hypothesis of null association.

To test the validity of the MDR method, we further conducted a logistic regression analysis that combined the two individually significant polymorphisms rs4961 and rs4343, the confounding factors (sedentary lifestyle, smoking, and alcohol habits), and the best interaction MDR model (diabetes, obesity, and *ADD1* rs4961) (Table 5). The results confirmed that the interaction between diabetes, obesity, and *ADD1* rs4961 showed a significant association of 10-fold increased EH risk (95% CI: 2.336–43184; $p = 0.002$) (Table 5). Furthermore, sedentary lifestyle and alcohol presented a risk for EH of 1.229 and 1.390, respectively, with statistical significance ($p < 0.05$).

TABLE 3. RISK PREDICTION OF FOURTEEN POLYMORPHISMS RELATED TO ESSENTIAL HYPERTENSION IN OUR POPULATION ($N = 1641$), SELECTED FROM THE BEST GENETIC MODEL OF INHERITANCE

Gene	SNP	Chr	Position	Cases	Controls	OR ^a (95% CI)	p Value	MAF (%)	p Value HW
<i>ACE</i> I/D	rs4340	17	61,565,892	65.7	62.8	1.137 (0.985–1.312) ^b	0.079 ^b	34.3	0.110
<i>ACE</i> 2350 A/G	rs4343	17	63,488,670	58.4	55.1	1.148 (0.999–1.321) ^c	0.053 ^c	41.6	0.721
<i>AGT</i> T/M (T174M)	rs4762	1	230,710,231	30.7	29.6	1.198 (0.941–1.525) ^d	0.143 ^d	30.7	0.790
<i>AGT</i> M/T (M235T)	rs699	1	230,710,048	43.5	42.1	1.117 (0.909–1.373) ^d	0.291 ^d	43.5	0.766
<i>AT1R</i> A/C	rs5186	3	148,742,201	24.8	24.0	1.094 (0.900–1.331) ^d	0.366 ^d	24.8	0.097
<i>CYP11B2</i> C/T	rs1799998	8	142,918,184	55.5	55.5	0.927 (0.724–1.187) ^d	0.548 ^d	44.5	0.823
<i>CYP17A1</i> T/C	rs11191548	10	103,086,421	10.7	10.3	1.150 (0.473–2.794) ^c	0.757 ^c	10.7	0.629
<i>ADD1</i> G/T	rs4961	4	2,904,980	16.3	14.7	2.379 (1.135–4.985) ^f	0.018 ^f	16.3	0.550
<i>GNB3</i> C/T	rs5443	12	6,845,711	40.7	38.3	1.275 (1.042–1.559) ^d	0.018 ^d	40.7	0.140
<i>ADRB1</i> R/G (R389G)	rs1801253	10	114,045,297	30.7	29.6	1.089 (0.897–1.322) ^d	0.387 ^d	30.7	0.213
<i>ADRB2</i> R/G (R16G)	rs1042713	5	148,826,877	55.8	57.8	0.925 (0.805–1.062) ^b	0.268 ^b	44.2	0.716
<i>SCNN1G</i> A/G	rs5718	16	23,182,544	56.8	55.7	1.170 (0.926–1.478) ^d	0.187 ^d	43.2	0.014
<i>SLC4A2</i> C/T	rs2303934	7	150,767,540	3.2	2.8	1.168 (0.769–1.775) ^d	0.467 ^d	3.2	0.021
<i>ATP2B1</i> A/G	rs2681472	12	89,615,182	15.3	15.1	0.780 (0.433–1.405) ^f	0.407 ^f	15.3	0.745

^aOR from the best model.

^bMultiplicative model.

^cAdditive model.

^dDominant model.

^eCodominant model.

^fRecessive model.

OR, odds ratio; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; HW, Hardy–Weinberg using $p < 0.004$ after Bonferroni correction; CI, confidence interval.

TABLE 4. BEST MODELS TO ANALYZE GENE-ENVIRONMENT INTERACTIONS BY MULTIFACTORIAL DIMENSIONALITY REDUCTION

Best model	Training balanced accuracy	Training odds ratio	Training p-value	Testing balanced accuracy	Testing odds ratio	Testing p-value	CV consistency
Obesity	0.620	3.682 (2.855–4.748)	<0.0001	0.620	3.682 (1.717–7.900)	0.0005	10/10
Diabetes; obesity	0.635	3.726 (2.936–4.728)	<0.0001	0.635	3.726 (1.823–7.614)	0.0002	10/10
Diabetes; obesity; <i>ADD1</i> ^a	0.638	3.725 (2.945–4.711)	<0.0001	0.638	3.725 (1.842–7.535)	0.0002	10/10
Diabetes; obesity; smoking; <i>ADD1</i>	0.639	3.952 (3.105–5.030)	<0.0001	0.628	3.467 (1.704–7.055)	0.0004	6/10

^aOverall best combination model with highest training balanced accuracy, highest testing accuracy, and best CV consistency. CV, cross validation.

Smoking appeared with a protective effect for EH (OR = 0.615 95% CI 0.485–0.780; $p < 0.0001$).

Because obesity appeared to be an important risk factor for EH development in our MDR analysis, we have further done a crossover analysis (Table 6) and a stratified analysis (Table 7) to investigate whether the obesity or *ADD1* rs4961 represented the greatest effect on the EH risk.

Results showed that both obesity and the TT genotype of *ADD1* rs4961 greatly increased the susceptibility to hypertension, but obesity seems to have the major effect (OR = 5.936 vs. OR = 2.638) (Table 6).

In the stratified analysis, results showed that on obese individuals, the *ADD1* rs4961 TT genotype had no significant association with EH ($p = 0.546$). On the other hand, the TT genotype showed a significant increase in EH risk in non-obese individuals (OR = 2.648, 95% CI: 1.125–6.236; $p = 0.021$), indicating that the risk for this polymorphism is greater when the person is nonobese (Table 7). The effect of obesity is much stronger than the existence of the *ADD1* variant; however, an interaction between the two variables synergistically increases the risk of EH.

Discussion

It is well established that both genetic and environmental factors contribute to the regulation and maintenance of BP. With the beginning of the human genome project and the International HapMap Project, SNPs have become increasingly prominent in studies of both multifactorial and multi-genomic diseases (Kuneš and Zicha, 2009).

In the present work, there were three polymorphic variants, which were significantly associated to EH individually—

rs4961, rs5443, and rs4343, this last on the threshold of significance. *ADD1* rs4961 was selected in the three-factor model along with obesity and diabetes as the best association model to predict EH. The four-factor model that included obesity, diabetes, *ADD1* rs4961, and *ACE* rs4343 was also selected but had a slightly lower testing balanced accuracy and a weak cross-validation consistency.

The *ADD1* G614T polymorphism (rs4961) results in the amino acid substitution of glycine by tryptophan (Gly460Trp), which is reported to be associated with a salt-sensitive form of hypertension. Cusi *et al.* (1997) found significant linkage of the alpha-adducin locus to EH ($p = 0.0003$) and greater sensitivity to changes in sodium balance among patients with the mutant allele, suggesting that alpha-adducin is associated with a salt-sensitive form of EH (Cusi *et al.*, 1997). However, epidemiological studies have shown that the contribution of *ADD1* Gly460Trp mutation to hypertension varies among different ethnic groups. A positive association of this genetic variant with EH has been confirmed in some studies (Tamaki *et al.*, 1998; Barlassina *et al.*, 2000; Ju *et al.*, 2003; Li *et al.*, 2012), but not in others (Larson *et al.*, 2000; Niu *et al.*, 2010).

As far as we know, this study is the first attempt to establish the interaction between polymorphic variants associated with EH and several environmental risk factors, in the susceptibility to develop EH, in a cohort of Southern European origin. The interaction between *ADD1* polymorphism and nongenetic risk factors, such as obesity and diabetes was identified as the overall optimum synergistic model by using a promising data-mining analytical method, MDR. This method has been successfully applied to detect high-order gene–gene and gene–environment interaction (Guy *et al.*, 2010).

TABLE 5. LOGISTIC REGRESSION ANALYSIS WITH MULTIFACTORIAL DIMENSIONALITY REDUCTION BEST MODEL AND CONFOUNDING VARIABLES

Variables	B	SE	Wald	df	Odds ratio (95% CI)	p Value
Smoking	−0.487	0.121	16.105	1	0.615 (0.485–0.780)	<0.0001
Sedentary life	0.206	0.101	4.190	1	1.229 (1.009–1.497)	0.041
Alcohol	0.329	0.104	9.957	1	1.390 (1.133–1.705)	0.002
<i>ADD1</i> (GT + TT) × Diabetes (Yes) × Obesity (Yes)	2.307	0.744	9.611	1	10.044 (2.336–43.184)	0.002
Constant	−0.081	0.086	0.891	1	0.922	0.345

Forward Wald method (SPSS vs. 19.0) with all variables staying in the model. Statistically significant for $p < 0.05$.

B, beta coefficient; SE, standard error; df, degrees of freedom.

TABLE 6. CROSSOVER ANALYSIS BETWEEN OBESITY AND ADD1 GENOTYPES

Obesity	ADD1	Cases	Controls	OR (95% CI)	p Value
No	GG	373	492	— ^a	— ^a
	GT	133	178	0.986 (0.759–1.281)	0.913
	TT	16	8	2.638 (1.117–6.230)	0.022
Yes	GG	223	78	3.771 (2.819–5.044)	<0.0001
	GT	94	35	3.543 (2.349–5.342)	<0.0001
	TT	9	2	5.936 (1.275–27.635)	0.010

Statistically significant for $p < 0.05$.

^aOR and p -values obtained by the genotype frequency using the ADD1 GG genotype as reference among the nonobese individuals.

Such best interaction cannot be overlooked because in obesity there are a variety of endocrine, genetic, and metabolic mechanisms linked to each other that include insulin resistance, hyperinsulinemia, increased serum aldosterone levels, salt sensitivity, and expanded plasma volume with increased peripheral vascular resistance, which can lead to EH. Since BMI represents the internal metabolic and physiological environment that plays a key role in the development of high BP (Feng *et al.*, 2012), and ADD1 rs4961 is one of the most important targets for salt sensitivity and expanded plasma volume, it is not surprising that their interaction may play an important role in the susceptibility to hypertension. However, the exact pathophysiological mechanisms that contribute to this association require further study.

On the other hand, it is now established that insulin resistance, which predicts type 2 diabetes, also has a role in the development of hypertension (Sowers, 2004; Conen *et al.*, 2007). Indeed, hypertension and diabetes substantially share common pathways, such as obesity, inflammation, oxidative stress, and insulin resistance.

In our case-control study, diabetes, obesity, alcohol consumption, and ADD1 TT genotype were confirmed as independent risk factors for EH by multivariate logistic regression. Curiously, smoking habits appear to be a protector factor. A likely explanation of these apparent protective effects of smoking on hypertension might be related to the fact that physicians had likely advised their hypertensive patients to quit smoking, so that the proportion of smoking hypertensives is lower than the proportion of nonsmoking ones.

Further research on gene-gene and gene-environment interaction with well-designed studies containing large sample size and multiple genetic polymorphisms and environmental risk factors are needed to provide more precise evidence for the issue.

TABLE 7. STRATIFIED ANALYSIS WITH THE INTERACTION BETWEEN OBESITY AND ADD1 GENOTYPES

Obesity	ADD1	Cases	Controls	OR (95% CI)	p Value
No	GG	373	492		
	GT	133	178	2.648 (1.125–6.236)	0.021
	TT	16	8		
Yes	GG	223	78		
	GT	94	35	1.604 (0.341–7.536)	0.546
	TT	9	2		

OR and p -values obtained by the recessive model (TT vs. GG + GT). Statistically significant for $p < 0.05$.

Strength and limitations

One limitation of the study was that only 13 polymorphisms of relevant genes for EH were analyzed, and it is of added interest to explore other candidate genes within different metabolic axes and other emerged from GWAS with yet unknown pathophysiological mechanisms.

Other limitation to the approach is that MDR method does not have a way to adjust for covariate effects, such as age, gender, alcohol, and smoking status, an often necessary step to obtain an unconfounded SNP interactions outcome. For this reason, we used a second logistic regression with the MDR best model adjusted for confounded variables and so we were able to confirm the independence of this interaction.

A strength of our study was the specificity of our population, which is supposed to be a genetically homogeneous Southern European Caucasian population (Brehm *et al.*, 2003; Gonçalves *et al.*, 2005). The use of genetically isolated populations has been particularly valuable for mapping rare recessive disorders, or more complex disorders due to a relatively uniform genetic background of the populations (Kristiansson *et al.*, 2008). Some culturally and genetically isolated populations have a more similar way of living, eating habits, and natural environment that reduces environmental variation (Jorde and Wooding, 2004).

Conclusion

According to our findings, the evolution to EH may actually be affected by the interaction of ADD1 rs4961, obesity, and type 2 diabetes in our population. In patients carrying this polymorphism, involved in salt handling, it will be of utmost importance the modification of lifestyle.

Acknowledgment

This study was supported by the European Regional Development Fund's Operational Program for the Enhancement of Economic Potential and Territorial Cohesion for the Autonomous Region of Madeira (INTERVIR+).

The authors are very grateful to Elsa Sousa who performed the phone calls to the subjects and all the administrative procedures.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Ana Célia Sousa, MD

Unidade de Investigação

Hospital Doutor Nélito Mendonça

Avenida Luís de Camões, no. 57

Funchal, Madeira 9004-514

Portugal

E-mail: anacelia.bett@gmail.com